Supplemental data for:

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Table S1. Affymetrix genechip analysis of genes that are uniquely down regulated by polyamide **1R-Chl**, and not affected by polyamide **1S-Chl** or by **Chl**.

Unique 1-Chl Downregulated Genes

	Affymetrix Probe	Relative Expression	Fold Change	Genbank ID	Name
1	205967_at	0.49	-2.03	NM_003542	histone 1, H4c
2	207060_at	0.62	-1.62	NM_001427	engrailed homolog 2
3	203998_s_at	0.62	-1.61	NM_005639	synaptotagmin I
4	209478_at	0.65	-1.54	NM_144998	stimulated by retinoic acid 13
5	201626_at	0.65	-1.53	BE300521	insulin induced gene 1
6	201195_s_at	0.65	-1.53	NM_003486	solute carrier family 7
7	212708_at	0.66	-1.52	AL049450	hypothetical protein LOC339287
8	209146_at	0.66	-1.52	BC010653	sterol-C4-methyl oxidase-like
9	204798_at	0.67	-1.49	NM_005375	v-myb myeloblastosis viral oncogene homolog
10	202294_at	0.68	-1.47	BC064699	propionyl Coenzyme A carboxylase, beta polypeptide
11	205542_at	0.68	-1.46	NM_012449	six transmembrane epithelial antigen of the prostate
12	205258_at	0.69	-1.46	NM_002193	inhibin, beta B (activin AB beta polypeptide)
13	218281_at	0.69	-1.45	NM_016055	mitochondrial ribosomal protein L48
14	218579_s_at	0.70	-1.43	NM_021931	DEAH (Asp-Glu-Ala-His) box polypeptide 35
15	205199_at	0.70	-1.43	NM_001216	carbonic anhydrase IX
16	218005_at	0.70	-1.42	BC041139	zinc finger protein 22 (KOX 15)
17	203860_at	0.71	-1.42	NM_000282	propionyl Coenzyme A carboxylase, alpha polypeptide
18	209303_at	0.71	-1.41	NM_002495	NADH dehydrogenase (ubiquinone) Fe-S protein 4
19	203614_at	0.71	-1.41	NM_021645	KIAA0266 gene product
20	222258_s_at	0.72	-1.39	NM_014521	SH3-domain binding protein 4
21	221908_at	0.73	-1.37	AK094682	hypothetical protein FLJ14627
22	216384_x_at	0.74	-1.36	AF170294	prothymosin a14 mRNA
23	218421_at	0.75	-1.33	NM_022766	ceramide kinase

Table S2. Polyamide **1R** binding sites in the histone H4c gene: in vitro assays. Binding affinities were determined by quantitative DNase I footprinting (with polyamide **1R**), and alkylation by thermal cleavage (with polyamide **1R-Chl**) and the radiolabeled H4c PCR product. The alkylated nucleotides are shown in red.

Sequence	K_a (nM)	Alkylation
5′-tg**aggaga**ct-3′ 3′-ac**tcctct**ga-5′	>100	no
5′-cg**aggtgt**gc-3′ 3′-gc**tccac**a**c**g-5′	0.7	yes
5′-cg**tcacct**at-3′ 3′-gc**agtgga**ta-5′	0.3	no
5′-gg**tctcct**ga-3′ 3′-cc**agagga**ct-5′	>100	no

Figure S1. Cell viability and cell numbers for polyamide **1R-Chl**-treated (A) and **Chl**-treated (B) SW620 cells. Viability was measured with an ATP metabolic assay (ApoSensor). The average values for two determinations, relative to the untreated control cells, are shown for each experimental condition. Cells were treated with polyamide or **Chl** for 4 days prior to analysis.

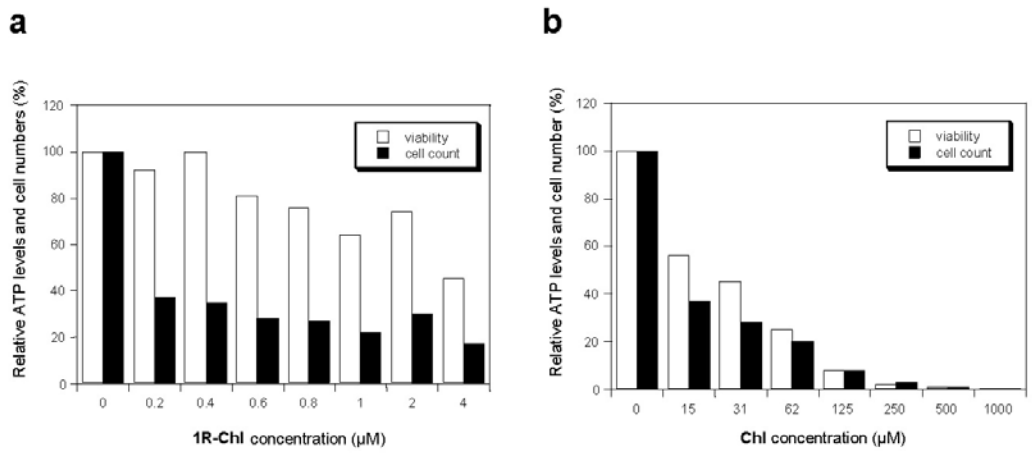


Figure S2. Deconvolution microscopy of **1R-bodipy** and **1S-bodipy** in unfixed SW620 colon carcinoma cells. Cells were incubated with 0.5 μM polyamide for 24 h prior to microscopy. The bar indicates 10 μ. The corresponding phase micrographs of the same cells are shown below, indicating nuclear localization of the fluorescent polyamides.

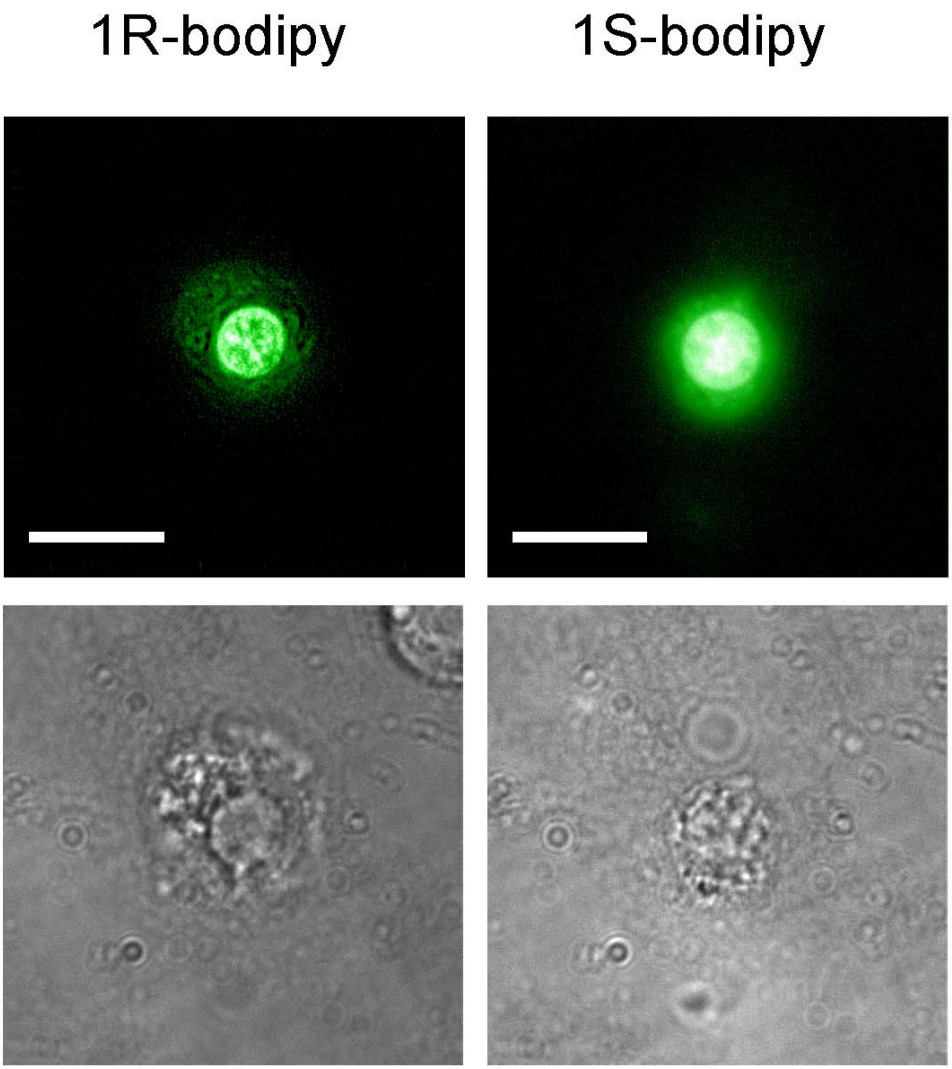
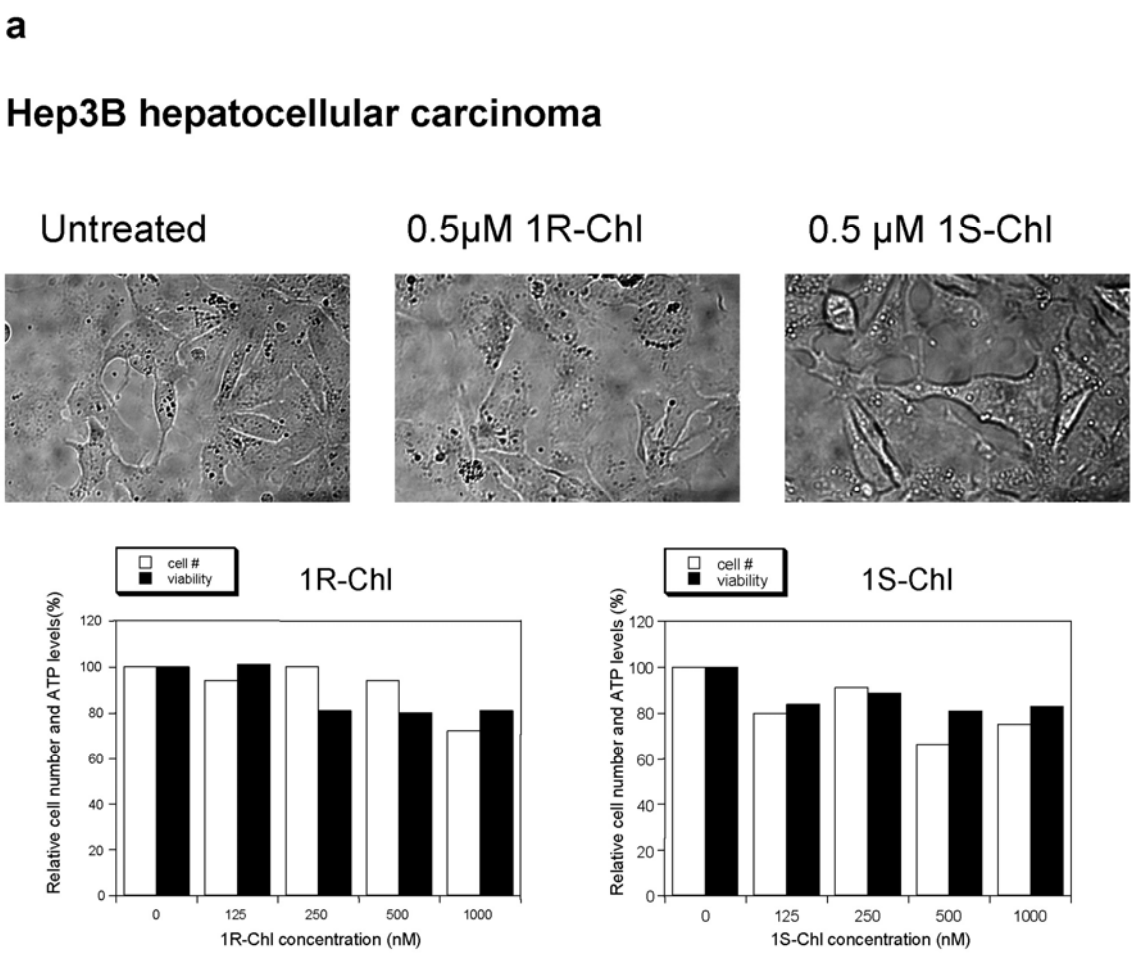
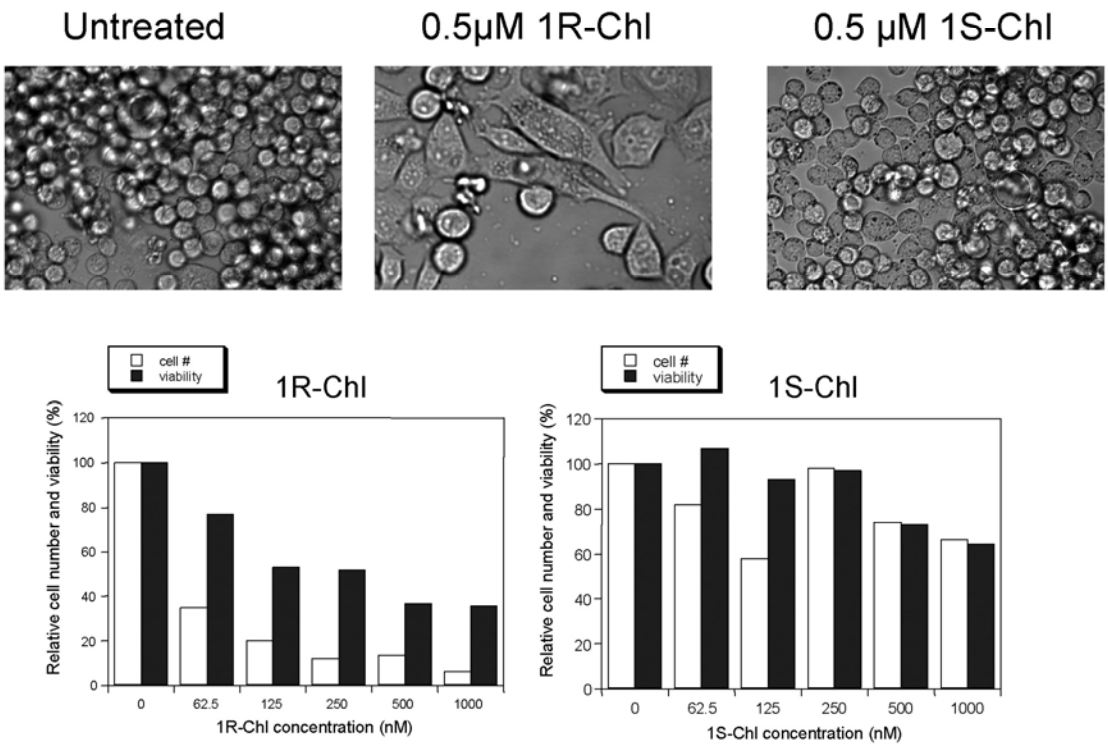


Figure S3. Effects of Polyamides **1R-Chl** and **1S-Chl** on Cell Growth, Morphology and Viability (A) Hep3B cells; (B) HeLa cells; (C) K562 lymphoid cells. Cells were incubated with the indicated concentration of **1R-Chl** or **1S-Chl** for 4 days prior to phase microscopy. All images are shown at the same magnification. Cell numbers were determined and viability was measured with an ATP assay (ApoSensor). Values are expressed relative to the no polyamide control and are the average of two determinations.



b

HeLa cells


Untreated
0.5μM 1R-Chl
0.5 μM 1S-Chl
cell #
viability
1R-Chl
Relative cell number and viability (%)
120
100
80
60
40
20
0
0
62.5
125
250
500
1000
1R-Chl concentration (nM)
cell #
viability
1S-Chl
Relative cell number and viability (%)
120
100
80
60
40
20
0
0
62.5
125
250
500
1000
1S-Chl concentration (nM)

c

K562 CML cells